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Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil

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Abstract

Biosorption of Pb(II) and Cu(II) ions from aqueous solutions has been studied in a batch system by using a bacterial strain isolated from metal polluted soil. The bacterial strain was identified as *Bacillus* sp. The optimum conditions of biosorption were determined by investigating the initial pH, contact time and the initial concentrations of metal ions at constant temperature (25 °C). The maximum biosorption of the metal ions was observed at pH 3.0 ± 0.1 for Pb(II) and pH 5.0 ± 0.1 for Cu(II) ions. Biosorption equilibrium times for Pb(II) and Cu(II) ions were observed in 15 and 30 min, respectively. The maximum biosorption capacities of Pb(II) and Cu(II) ions on *Bacillus* sp. were determined to be 92.27 ± 1.17 mg g^{-1} at 250 mg l^{-1} concentration and 16.25 ± 1.64 mg g^{-1} at 200 mg l^{-1} concentration, respectively. The experimental adsorption data were fitted to Langmuir isotherm model. Competition of metal ions during biosorption was investigated in binary metal solutions. The interactions between metal ions and functional groups on the cell wall surface of the biomass were confirmed by FTIR, SEM and EDAX analysis. The results indicated that bacterial isolate *Bacillus* sp. is a suitable biosorbent for the removal of Pb(II) and Cu(II) ions from aqueous solutions.

Keywords: *Bacillus* sp.; Biosorption; Competitive biosorption; Cu(II); Langmuir isotherm; Pb(II)

1. Introduction

Discharge of heavy metals from various industries such as mining, ore processing, smelting and metal plating can easily create metal pollution and causes hazardous effects on humans, animals and environmental balances. Lead and copper are among those metals widely used in industry and their accumulation in the living tissues may cause serious health problems [1]. While lead is extremely toxic and can damage the nervous system, kidneys and reproductive system, particularly in children, high levels of copper can cause toxic effects like all other heavy metals although copper is an essential trace element. The EPA requires lead and copper in drinking water not to exceed 0.015 and 1.3 mg l^{-1}, respectively [2].

The severe toxic effects imposed by heavy metals on living tissues and environment directed the research at investigating alternative technologies for wastewater purification systems. Conventional separation techniques applied to the treatment of industrial effluents include chemical precipitation, chemical oxidation or reduction, filtration, ion exchange and electrochemical processes. However, technical and economical constraints encountered in the application of these traditional methods have directed attention to the search for new technologies involving metal removal from waste streams [3,4].

As a result of development in the field of environmental microbiology, recent studies have focused on the use of microbial-based potential biosorbents such as yeast, bacteria and fungi. They are capable of removing heavy metals from waste streams [5–9]. This biological phenomenon defined as biosorption seem to be a good alternative to the existing methods since it does not produce chemical sludges, it could be highly selective, more efficient, easy to operate and hence cost effective for the treatment of large volumes of wastewaters containing low pollutant concentrations [10]. The biosorption process includes physicochemical interactions between metal ions and several anionic ligands present on the biomass like carboxyl, phosphoryl, carbonyl and sulfhydryl [11]. The efficiency of this process depends on various factors varying from the type of metal ion being studied to the type of biosorbent material used as well as pH [12].

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Soil is a dynamic, multi-component system, whose properties depend upon by microbial, chemical, hydrological and geological processes in it. The interaction between microorganism and metal ions, in soil, is considered to be the result of metal competition by all the other components in the system. Life in a polluted environment get difficulties for the microorganisms in many ways, meanwhile they have greater energy demands in order to cope with the toxicity of pollutants. The ability to grow even at high metal concentrations is found in many microorganisms and may be the result of intrinsic or induced mechanisms, as well as other environmental conditions such as pH, speciation, redox, etc., which can reduce metal toxicity [13]. Recent studies indicated that the microbial strains isolated from contaminated soils have excellent removal capacity of heavy metals from aqueous solutions [14].

The objective of this study was to isolate microbial strains from soil contaminated with copper and lead ions, identify and study the biosorption potential of the appropriate strains towards copper and lead. The metal loading capacity of bacterial biomass was determined as a function of initial pH, contact time and initial metal ion concentration. The monocomponent biosorption data have been analyzed by Freundlich and Langmuir isotherm models. The biosorption performance of bacterial biomass in binary metal mixtures was also investigated. The role played by the functional groups present in the biomass in biosorption process was examined by FTIR, SEM and EDAX analysis in addition to the environmental parameters affecting the biosorption process.

2. Materials and methods

2.1. Isolation and screening of microorganism

The microorganism used in this study was isolated from metal polluted soil. The soil samples were initially collected from natural environment and 100 g of soil was polluted with Pb(II) and Cu(II) by treating with 250 ml of 1000 mg l^{-1} $Pb(NO_3)_2$ and $CuSO_4{\cdot}5H_2O$ solutions in 1000 ml flask to select the appropriate bacterial strains having greater resistance towards Pb(II) and Cu(II) toxicity. Soil sample was stirred with metal solutions overnight and then liquid phase was separated by filtration. For isolation of bacterial strains 10 g of this soil sample was homogenized with 90 ml of 0.85% (w/v) sterile saline solution. Serial dilutions were prepared in 9 ml of 0.85% (w/v) sterile saline solution and plated on nutrient agar (NA) (Merck). From the NA plates, colonies representative of all different morphologies were chosen at random, purified by sub-culturing and maintained in a slants of NA. All culture works were conducted aseptically.

For preliminary tests, five strains of bacteria were originally isolated from polluted soil in the laboratory and investigated for biosorption capacities of dried biomass. Equal and known quantities of microorganisms added to Erlenmeyer flask containing metal solutions of known concentrations. The flasks were agitated at 125 rpm on an orbital shaker for 30 min. At the end of the contact time, biomass was separated from the reaction mixtures by filtration and the filtrate was analyzed for residual metal ion concentration.

During preliminary screening tests, the bacterium (isolate 1) demonstrated better biosorption capacity for both metals than the others.

2.2. Identification and preparation of effective isolate

Microscopic and biochemical tests were applied to effective bacterial isolate according to Bergey's Manual of Systematic Bacteriology [15] and The Prokaryotes [16].

The bacterial isolate was incubated at 30 °C and 125 rpm for 24 h in the nutrient broth (Merck) at neutral pH. The biomass was separated from the growth medium by centrifugation at 4500 rpm for 10 min and washed throughly distilled water. It was spread on Petri dishes and then dried at 80 °C for overnight. Dried biomass was powdered using mortar and pestle. The powdered biomass was sieved to select particles 150 μm for use as a biosorbent in batch studies.

2.3. Preparation of metal solutions

Metal solutions used in this study were prepared by dilution of 1000 mg l^{-1} stock solutions of Pb(II) and Cu(II) obtained by dissolving $Pb(NO_3)_2$ and $CuSO_4{\cdot}5H_2O$, respectively, in distilled and deionized water.

2.4. Biosorption experiments

All the batch biosorption experiments were carried out with 0.1 g of biosorbent at 25 °C in 250 ml Erlenmeyer flasks to elucidate the optimum operating conditions which enhance the Pb(II) and Cu(II) uptakes. The effect of pH on the biosorption capacitiy of the bacterial biomass for Pb(II) and Cu(II) was investigated in the pH range of 1.0–6.0 by using 50 ml solutions containing 100 mg l^{-1} of metal ions. The solutions were adjusted to required pH values using 1N H_2SO_4 and 1N NaOH and then not further controlled. Biosorbent was added to medium and the reaction mixture was shaken on an orbital shaker at 125 rpm for 120 min which is sufficiently long for sorption equilibrium. Experimental control tests were also carried out in the absence of the biosorbent to investigate the removal of dissolved Pb(II) and Cu(II) which might occur via chemical precipitation and sorption on to vessel walls. The effect of contact time on the biosorption was investigated in the time range of 5–120 min. For this purpose 3 ml of samples were taken from the biosorption media at definite intervals. Similarly above, metal solutions with varying concentrations (ranging from 5 to 300 mg l^{-1}) were used to assess the effect of initial metal ion concentration. In the latter experiments the pH of the Pb(II) and Cu(II) solutions was adjusted to 3.0 ± 0.1 and 5.0 ± 0.1, respectively (optimum pH values). The samples were centrifuged at 4500 rpm for 5 min and the supernatant liquid was analyzed for residual metal ion concentration.

Competitive biosorption of Pb(II) and Cu(II) ions was investigated from their solutions containing these two metals simultaneously. The initial concentrations of the competing metal ion were varied from 25 to 100 mg l^{-1} while the concentration of

the dominant metal ion in each biosorption medium was held constant at 25, 50 and 100 mg l^{-1}. The pH value of the biosorption media was adjusted to optimal value for dominant metal ion.

The amount of adsorbed metal ions per gram biomass was calculated by using the general equation:

$$q_e = \frac{(C_i - C_e)V}{M} \quad (1)$$

where q_e is the amount of metal ions adsorbed on the biomass (mg g^{-1}), C_i the initial metal ion concentration in solution (mg l^{-1}), C_e the final metal ion concentration in solution (mg l^{-1}), V the volume of the medium (l) and M is the amount of the biomass used in the reaction mixture (g).

2.5. Statistical analysis

All the experiments were carried out in triplicate and experimental errors were estimated and are depicted with error bars and standard deviations are indicated wherever necessary. All statistical analysis was done using SPSS 9.05 for Windows where it is possible to evaluate whether the effect and the interaction among the investigated factors are significant with respect to the experimental error.

2.6. Analysis of metal ions

The concentrations of unadsorbed Pb(II) and Cu(II) ions in the supernatant were determined by using an Atomic Absorption Spectrophotometer (Hitachi 180-70, Japan) with an air–acetylene flame. Deuterium background correction was used and the spectral slit width was 1.3 nm. The working currents/wavelengths for Pb(II) and Cu(II) were 7.5 mA/283.3 nm and 7.5 mA/324.8 nm, respectively. Calibration solutions prepared from atomic absorption stock solutions (998 ± 2 mg Pb(II) l^{-1} in HNO_3 0.5 mol l^{-1}, and 1001 ± 2 mg Cu(II) l^{-1} in HNO_3 0.5 mol l^{-1}, Sigma) were used for checking the instrument response for every 10 readings.

2.7. Biosorption characterization on the basis of bacterial surface

The chemical characteristics of effective isolate before and after metal sorption were analyzed and interpreted by FTIR spectroscopy. The spectra were recorded in a Bruker Tensor 27 Fourier transform infrared spectrometer with the samples prepared as KBr discs. All spectra were plotted using the same scale on the transmittance axis.

The surface structure of biosorbent was analyzed by scanning electron microscope (Cam Scan Oxford Link SEM) coupled with energy dispersive X-ray analysis (EDAX). The acceleration voltage was constant at 20 kV and the microprobe was focused at a magnification of 2000. Unloaded and metal-loaded biomass samples were mounted on a stainless steel stab with a double-stick tape followed by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs.

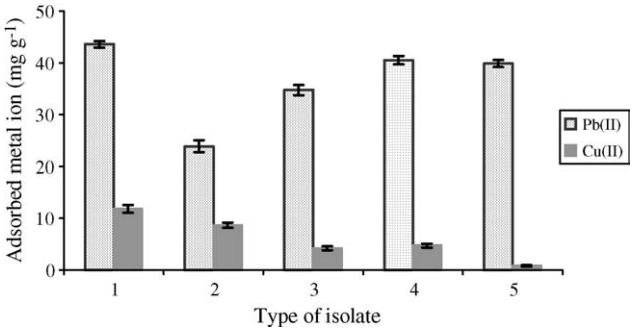

Fig. 1. Comparison of the biosorption capacities of isolates obtained from metal polluted soil. The bars represent the standard error of the mean (the difference in Pb(II) and Cu(II) biosorption capacities of the isolate 1 and the other isolates was found to be statistically significant ($p < 0.05$)).

3. Results and discussion

3.1. Selection of effective isolate

In this study, while the five morphologically distinct bacterial strain isolated, fungi and yeast were not isolated. As seen in Fig. 1, isolate 1 has maximum biosorption capacity for Pb(II) and Cu(II) when compared with the other isolates. Nonliving (dried) biomasses were used for comparison. Also the difference in biosorption ability of this isolate was found to be statistically significant from the others ($p < 0.05$). This most effective bacterial strain was identified according to Bergey's Manual of Systematic Bacteriology [15] and The Prokaryotes [16]. Biochemical tests performed for identification and microscopic properties of effective isolate are shown in Table 1. The bacterium was belonging to *Bacillus* genus according to these tests. In this article, the bacterial strain is named as *Bacillus* sp. (ATS-1).

The walls of gram positive bacteria are efficient metal chelators. The carboxyl group of the glutamic acid of peptidoglycan was the major site of metal deposition. Carbohydrate groups are important metal binding sites. Some *Bacillus* species produce carbohydrate capsules [16–18].

Table 1
Biochemical and microscopic characteristics of *Bacillus* sp. (ATS-1)

Properties	Result	Properties	Result
Gram reaction	+	Formation of Indole	–
Cell shape	Rod	Growth at pH 6.8, nutrient broth	+
Cell diameter > 1.0 μm	+	Growth at pH 5.7, nutrient broth	+
Spores round	–	Growth in NaCl 2%	+
Sporangium swollen	–	Growth in NaCl 5%	+
Catalase	+	Growth in NaCl 7%	–
Anaerobic growth	–	Growth in NaCl 10%	–
Voges–Proskauer test	–	Growth at 5 °C	–
Acid from D-glucose	+	Growth at 10 °C	–
Acid from D-xylose	–	Growth at 30 °C	+
Acid from D-mannitol	–	Growth at 40 °C	+
Hydrolysis of casein	–	Growth at 50 °C	–
Hydrolysis of gelatin	–	Growth at 55 °C	–
Hydrolysis of starch	+	Growth at 65 °C	–
Utilization of citrate	–		

(+): 90% or more strain positive; (–): 90% or more strain negative.

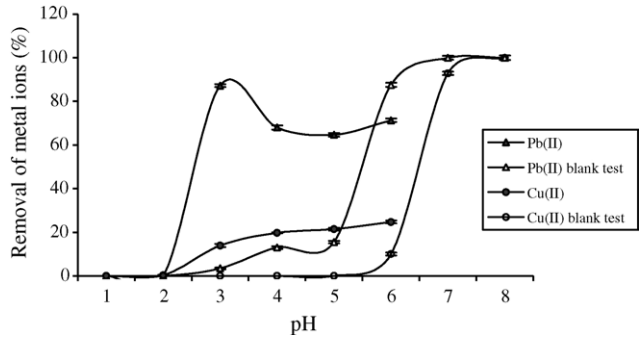

Fig. 2. Effect of pH on the metal biosorption by *Bacillus* sp. (ATS-1); 50 ml single metal solution (100 mg l^{-1}) of Pb(II) and Cu(II) was contacted with 0.1 g of biomass for 120 min. The bars represent the standard error of the mean.

3.2. Effect of pH on the biosorption

Earlier studies on heavy metal biosorption showed that pH was an important factor affecting the biosorption process [19,20]. The interaction of metal cations with the electron-rich functional groups located on the biomass may be strongly sensitive to the pH value of the environment. The inconsistency in literature regarding the influence of pH on biosorption seems to indicate that the pH would alter the sorption of metal ions and it may vary with the type of sorbents (biomass) and sorbates (metal ions) [21]. In the present study, the optimum initial pH values for biosorption of Pb(II) and Cu(II) ions were determined as 3.0 ± 0.1 and 5.0 ± 0.1, respectively (Fig. 2). The difference in biosorption capacities was found to be statistically significant at pH values 1.0, 2.0, 4.0, 5.0 and 6.0 comparing with pH 3.0 for Pb(II) ($p < 0.05$). At pH values between 4.0 and 6.0 biosorption capacity differ significantly from below pH 4.0 for Cu(II) ions ($p < 0.05$). Experimental control tests indicated that the removal mechanism is purely biosorption. As can be seen in Fig. 2, Pb(II) and Cu(II) ions become precipitate in the form of $Pb(OH)_2$ and $Cu(OH)_2$, respectively, because of increasing concentration of OH^- ions after the pH values 3.0 for Pb(II) and 5.0 for Cu(II). However, if this precipitation contributed to the Pb(II) and Cu(II) biosorption, the removal capacity should not have decreased and/or fixed at greater than these pH values.

The pH of the adsorption medium affects the solubility of metal ions and the ionization state of the functional groups on the cell wall of biosorbent. Because of high proton concentrations at extremely acidic conditions, cell wall functional groups are closely associated with the protons and it restricts the approach of metal ions as a result of increasing positive charge density of binding sites [22–24].

3.3. Effect of contact time on the biosorption

The effect of contact time on the biosorption of metal species (100 mg l^{-1}) on *Bacillus* sp. (ATS-1) was investigated at a constant temperature (25 °C). It has been determined that rapid adsorption of Pb(II) and Cu(II) ions was observed in initial 15 and 30 min, respectively (Fig. 3), and then the uptake of metal ions did not significantly change with contact time ($p > 0.05$).

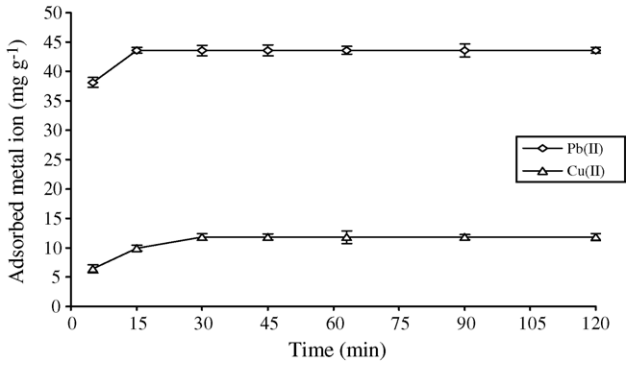

Fig. 3. Effect of contact time for Pb(II) and Cu(II) biosorption by *Bacillus* sp. (ATS-1); 50 ml single metal solution (100 mg l^{-1}) was contacted with 0.1 g of biomass at pH 3.0 for Pb(II) and 5.0 for Cu(II). The bars represent the standard error of the mean.

This rapid initial uptake of metals may be an important parameter for a practical application of biosorption in industrial wastewater treatment.

3.4. Effect of initial metal ion concentration on the biosorption

The effect of initial metal ion concentration on the biosorption capacity of *Bacillus* sp. (ATS-1) was studied at optimum pH values and contact time. These experiments were carried out using single metal ion solutions (5–300 mg l^{-1}). The amount of metal ions adsorbed per unit mass of bacterial biosorbent *Bacillus* sp. (ATS-1) increased first with increasing of the initial metal ion concentration and reached to a saturation value. Then the value did not change with the initial metal ion concentration ($p > 0.05$) (Fig. 4).

As seen from Fig. 4, the maximum biosorption capacities of *Bacillus* sp. (ATS-1) biomass for Pb(II) and Cu(II) ions were determined as 92.27 ± 1.17 mg g^{-1} at 250 mg l^{-1} initial Pb(II) concentration and 16.25 ± 1.64 mg g^{-1} at 200 mg l^{-1} initial Cu(II) concentration. Biosorption results of Pb(II) and Cu(II) reported by the other researchers in the literature by various biosorbents and operating conditions are summarized in Table 2.

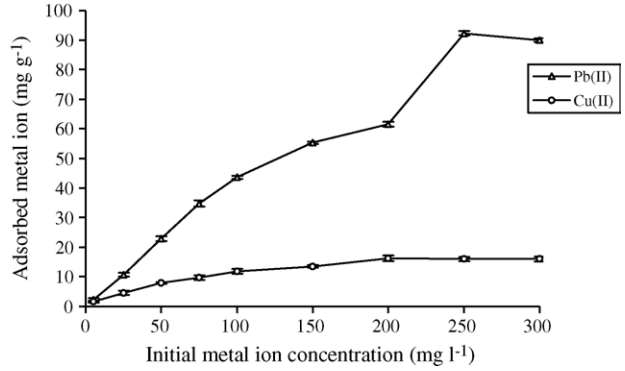

Fig. 4. Effect of initial metal ion concentration on the biosorption of Pb(II) and Cu(II) by *Bacillus* sp. (ATS-1); 50 ml single metal solution (5–300 mg l^{-1}) was contacted with 0.1 g of biomass at pH 3.0 for Pb(II) and 5.0 for Cu(II). The bars represent the standard error of the mean.

Table 2
Some biosorption results and operating conditions of Pb(II) and Cu(II) ions from the literature by various biosorbents

Metal type	Biosorbent type	Biosorption capacity ($mg\,g^{-1}$)	Operating conditions				
			pH	T (°C)	Initial metal ion concentration range ($mg\,l^{-1}$)	Biomass ($g\,l^{-1}$)	References
Pb(II)	*Phanerochaete chrysosporium* (formaldehyde and alkali pretreated)	12.34	4.5	27	50	2	[28]
	Streptomyces longwoodensis	100	3.0	28	50–200	0.3	[29]
	Saccharomyces cerevisiae	2.7	5.0	25	10.4	2	[30]
	Rhizopus arrhizus	85.6	5–7	–	10–600	3	[31]
	S. noursei	36.5	6.1	30	2–207	3.5	[32]
	Mucor rouxii	17.13	5.0	–	10	–	[33]
	Pinus sylvestris	11.38	4.0	25	10–100	4	[34]
	S. rimosus (NaOH treated)	135	2–12	–	500	3	[35]
	Bacillus sp. (ATS-1)	92.27 ± 1.17[a]	3.0	25	250	2	This study
Cu(II)	*S. cerevisiae*	0.8	4.0	25	3.2	2	[30]
	S. noursei	9	5.5	30	6–65	3.5	[32]
	Aureobasidium pullulans	6	5.5	25	1–320	1	[36]
	Melanin of *Aureobasidium pullulans*	9	5.5	25	1–320	1	[36]
	Cladosporium resinae	16	5.5	25	1–320	1	[36]
	Melanin of *C. resinae*	25.4	5.5	25	1–320	1	[36]
	Aspergillus niger 405	4.4	5.0	25	10	10	[37]
	R. arrhizus	10.76	4.5	30	75	1.32	[38]
	A. niger	9.53	5.0	30	75	1.28	[38]
	Bacillus sp. (ATS-1)	16.25 ± 1.64[a]	5.0	25	200	2	This study

[a] Standard deviation of the mean.

The uptake values obtained in this study are comparable and were found to be higher than that of many corresponding biosorbents.

3.5. Adsorption isotherms

The biosorptive metal uptake can be quantitatively evaluated by experimental equilibrium isotherms. The graphical expression of isotherm is a plot of the metal uptake by the per unit weight of biosorbent against the residual metal ion concentration in the biosorption medium. There are two widely accepted and easily linearized adsorption isotherm models used in the literature, which are namely Freundlich [25] and Langmuir [26] models.

The Freundlich model based on the relationship between the metal uptake capacity "q_e" ($mg\,g^{-1}$) of biomass and the residual (equilibrium) metal ion concentration "C_e" ($mg\,l^{-1}$). The general Freundlich equation is as follows:

$$q_e = K_f C_e^{1/n} \tag{2}$$

and linearized form of this model is

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \tag{3}$$

where intercept, $\ln K_f$, is a measure of adsorbent capacity, and the slope, $1/n$, is the intensity of adsorption.

The general Langmuir equation is commonly presented as

$$q_e = \frac{Q_0 b C_e}{1 + b C_e} \tag{4}$$

and the equation may be linearized as follows:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b} + \frac{C_e}{Q_0} \tag{5}$$

where q_e is the amount of metal ion removed ($mg\,g^{-1}$), C_e the equilibrium concentration ($mg\,l^{-1}$), Q_0 and b are the Langmuir constants related to adsorption capacity and affinity, respectively. The Freundlich and Langmuir constants, have been calculated from the corresponding plots (Figs. 5 and 6) for the biosorption of Pb(II) and Cu(II) on the biosorbent and the results are presented in Table 3. The regression coefficients obtained for Pb(II) from the Freundlich and Langmuir models were 0.886 and 0.920, respectively. For Cu(II) ions, the regression coefficients for the Freundlich and Langmuir models were 0.982 and 0.986, respectively. Langmuir equation provides a better correlation with the experimental data. Therefore, biosorption process in

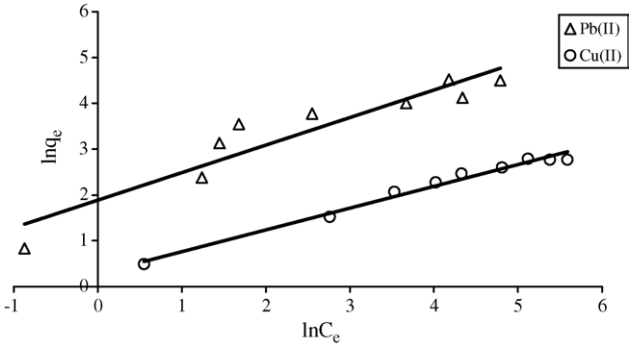

Fig. 5. Freundlich adsorption isotherms for Pb(II) and Cu(II) biosorption by *Bacillus* sp. (ATS-1).

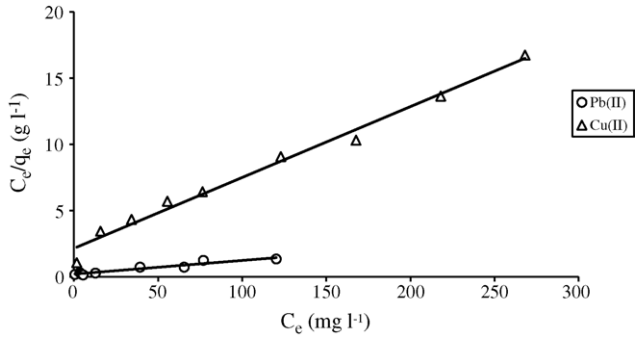

Fig. 6. Langmuir adsorption isotherms for Pb(II) and Cu(II) biosorption by *Bacillus* sp. (ATS-1).

Table 3
Freundlich and Langmuir isotherm constants and regression coefficients for Pb(II) and Cu(II) biosorption at optimum conditions in single metal ion systems

Metal ion	Freundlich			Langmuir		
	K_f	n	R^2	Q_0	b	R^2
Pb(II)	6.59	1.66	0.886	96.15	0.055	0.920
Cu(II)	1.33	2.1	0.982	18.69	0.025	0.986

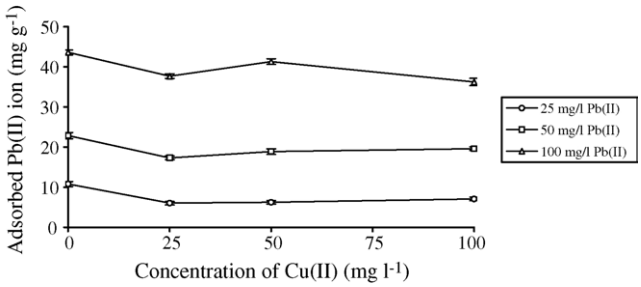

Fig. 7. Comparison of the effect of initial Pb(II) ion concentration on the initial adsorption capacities of Pb(II) ions to *Bacillus* sp. (ATS-1), with Pb(II) ions present as the single metal and in the presence of increasing concentrations of Cu(II) ions at pH 3.0 (biomass 0.1 g, temperature 25 °C, agitation rate 125 rpm, 15 min incubation). The bars represent the standard error of the mean.

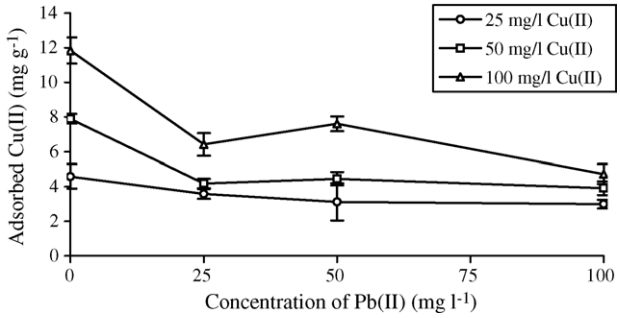

Fig. 8. Comparison of the effect of initial Cu(II) ion concentration on the initial adsorption capacities of Cu(II) ions *Bacillus* sp. (ATS-1), with Cu(II) ions present as the single metal and in the presence of increasing concentrations of Pb(II) ions at pH 5.0 (biomass 0.1 g, temperature 25 °C, agitation rate 125 rpm, 30 min incubation). The bars represent the standard error of the mean.

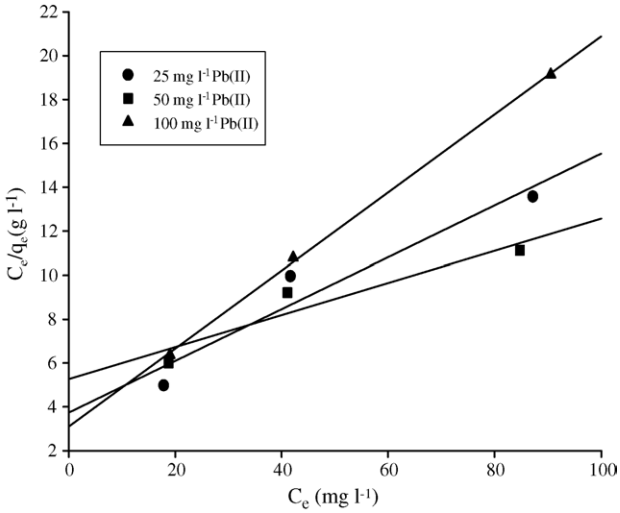

Fig. 9. Langmuir isotherm plots for Pb(II) biosorption in the presence of increasing Cu(II) concentrations at pH 3.0. (biomass 0.1 g, temperature 25 °C, agitation rate 125 rpm, 30 min incubation).

this study may be interpreted as monolayer adsorption. Greater values of b indicate the affinity of biosorbent to investigated metals and imply strong binding of metal ions. Consequently, the preference of investigated biosorbent for metal ions is as follows: Pb(II) > Cu(II).

3.6. Competitive biosorption

In the single-metal ion situation, the uptake capacity of biomass obtained with initial concentrations of Pb(II) ions at 25, 50 and 100 mg l^{-1} was found to be 10.78 ± 1.14, 22.87 ± 1.31 and 43.60 ± 1.09 mg g^{-1}, respectively, while the uptake values obtained in the binary metal solutions slightly reduced by Cu(II) ions (Fig. 7). However, the uptake values obtained in the binary metal solutions at 100 mg l^{-1} initial concentration of Pb(II) ions were found to be 37.68 ± 1.02, 41.29 ± 1.27 and 36.25 ± 1.61 mg g^{-1} when the initial concentrations of Cu(II) ions were 25, 50 and 100 mg l^{-1}, respectively.

The effects of the presence of Pb(II) ions on the biosorption of Cu(II) ions were investigated in the same manner. The results also indicated that the biosorption capacities of Cu(II)

Table 4
Langmuir isotherm constants for Pb(II) and Cu(II) biosorption in binary mixtures

Constants	Pb(II)			Cu(II)		
	25 mg l^{-1} Cu(II)	50 mg l^{-1} Cu(II)	100 mg l^{-1} Cu(II)	25 mg l^{-1} Pb(II)	50 mg l^{-1} Pb(II)	100 mg l^{-1} Pb(II)
Q_0	8.472	13.717	5.627	−9.533	−5.907	−59.524
b	0.032	0.014	0.057	−0.034	−0.050	−0.014
R^2	0.929	0.897	0.999	0.596	0.334	0.097

ions were reduced by Pb(II) ions similarly above. For example, in the single ion situation the uptake value obtained with the initial concentration of Cu(II) ions at 100 mg l^{-1} was found to be 11.84 ± 1.32 mg g^{-1}, while the uptake values obtained in the binary metal mixtures at the same initial concentration of Cu(II) ions were found to be 6.42 ± 1.12, 7.61 ± 0.75 and 4.73 ± 0.99 mg g^{-1} when the initial concentrations of Pb(II) ions were 25, 50 and 100 mg l^{-1}, respectively (Fig. 8). The results obtained from binary metal mixtures were also evaluated by the Langmuir isotherm model. The Langmuir constants for this system were presented in Table 4. According to R^2 values, the Langmuir equation provides a better correlation with the experimental data for Pb(II) biosorption in the presence of Cu(II) (Fig. 9). Since the Cu(II) biosorption in binary mixture was not followed by the Langmuir isotherm model, the plot is not shown as figure. The affinity order in binary metal mixtures was the same in single ion situation (Pb(II) > Cu(II)).

The previous studies indicated that biosorption yields for both metals in binary solutions lower than single metal solutions for one kind of ions and in the binary metal mixtures, both metal uptake and adsorption yield for one kind of ions decreased with increasing concentration of the other metal ions [27,28].

3.7. FTIR spectral analysis

The FT-IR spectra of unloaded and metal loaded *Bacillus* sp. (ATS-1) biomass in the range of 400–4000 cm^{-1} were taken to find out which functional groups are responsible for the biosorption process and presented in Fig. 10. As seen in this figure unloaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass. The spectrum pattern of unloaded biomass showed changes of certain bands in the region of 1546–531 cm^{-1} as compared to Pb(II) and Cu(II) loaded biomass. Band shifts were observed for the signals at

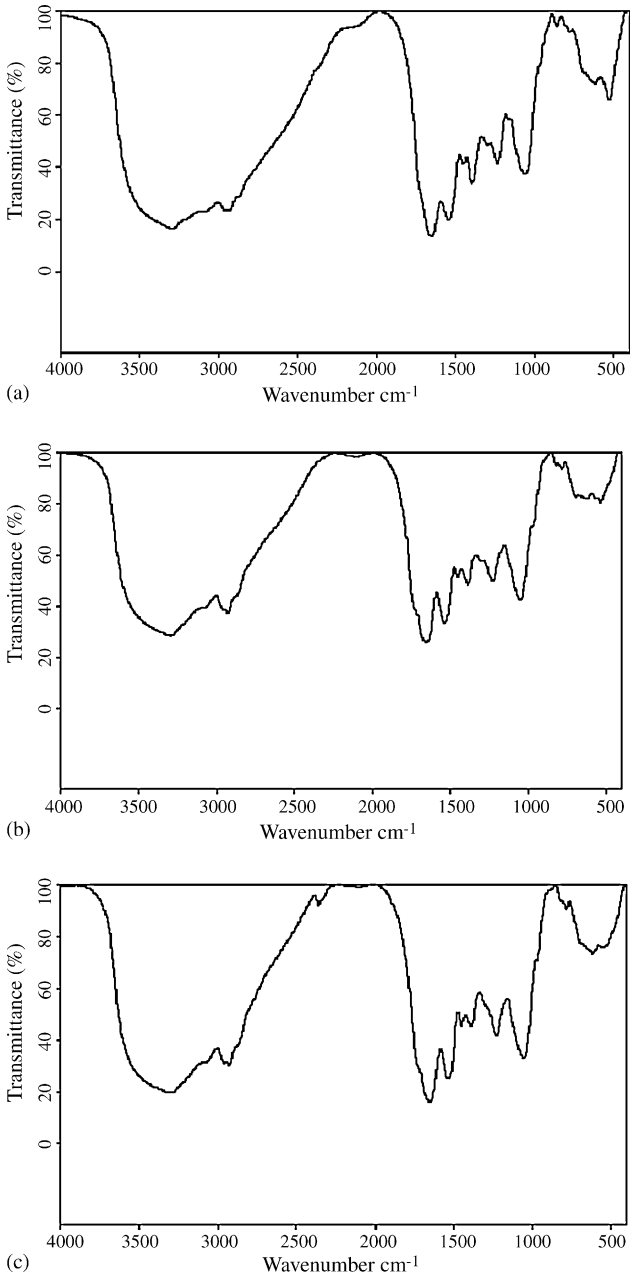

Fig. 10. FT-IR spectra of *Bacillus* sp. (ATS-1) (a) before metal loaded (b) after Pb(II) loaded and (c) after Cu(II) loaded.

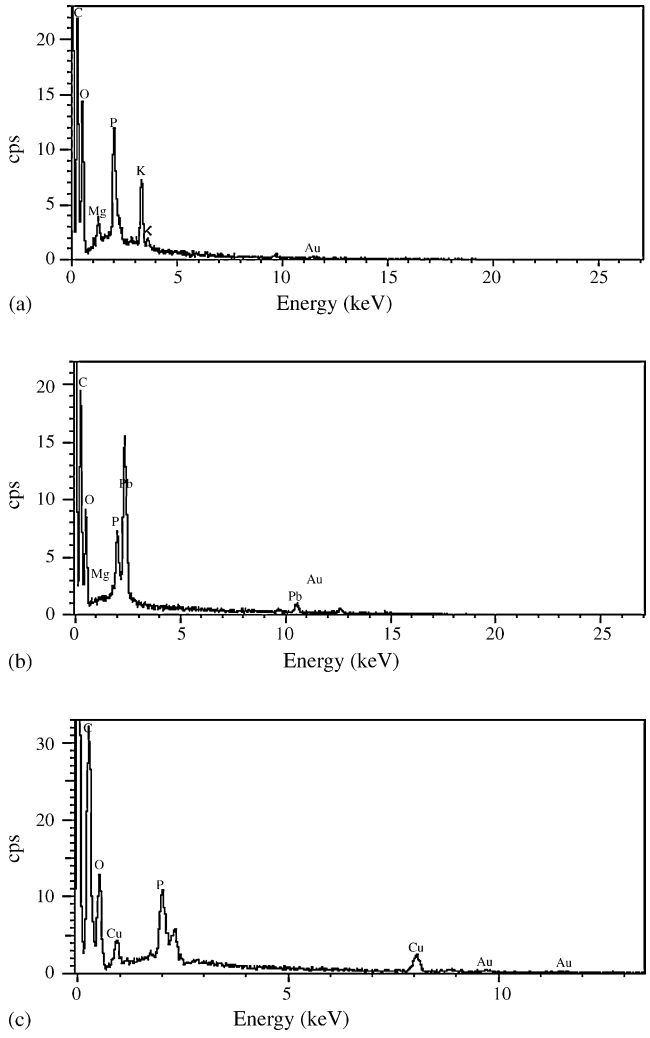

Fig. 11. Typical EDAX spectra of *Bacillus* sp. (ATS-1) (a) before metal loaded (b) after Pb(II) loaded and (c) after Cu(II) loaded.

1546 cm^{-1} (indicative of C–N stretching and N–H deformation), 1238 cm^{-1} (indicative of $–SO_3$ groups) and 1398 cm^{-1} (indicative of COO^- anions). There was also clear that aromatic –CH stretching peak at 861 cm^{-1} for unloaded biomass was disappeared after the sorption. Finally, it should be noted that intensity decrease with band shifts of the peaks in the region of lower wavenumbers (under 700 cm^{-1}) after metal sorption could be attributed to an interaction between the both metal ions and N-containing ligands. These changes observed in the spectrum indicated the possibly involvement in biosorption process of functional groups on the surface of the biomass.

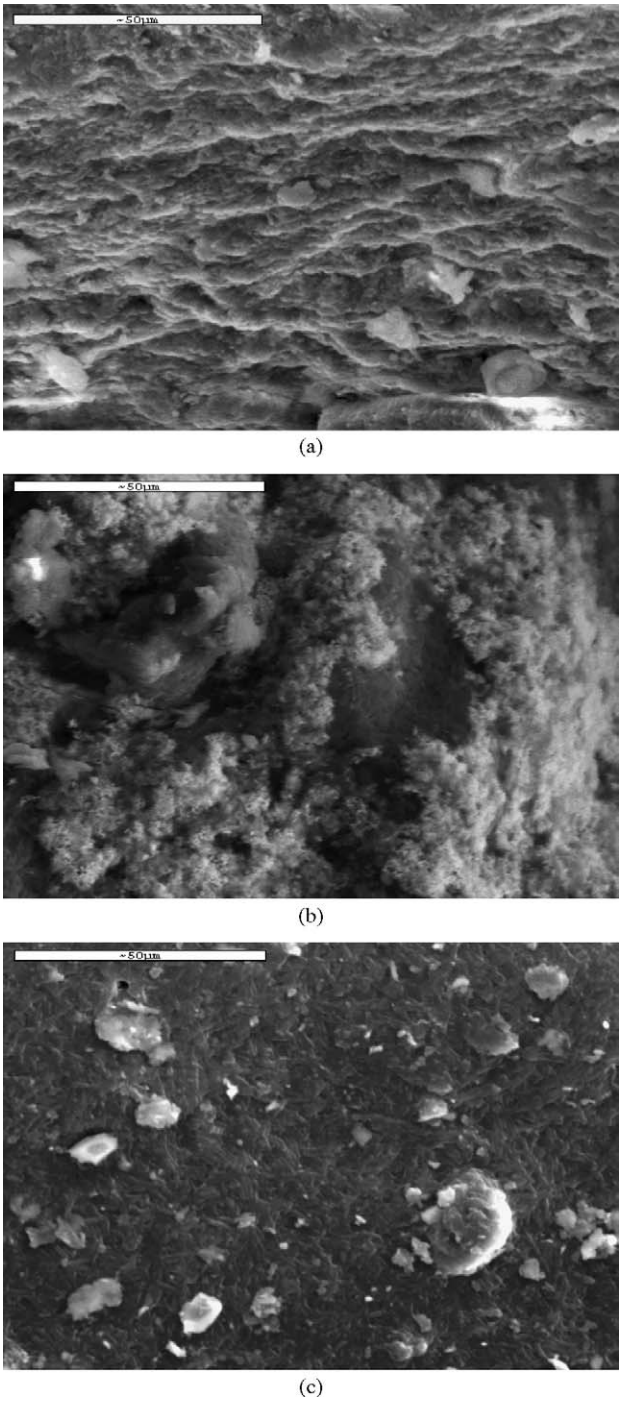

Fig. 12. Typical SEM micrographs of *Bacillus sp.* (ATS-1) (a) before metal loaded (b) after Pb(II) loaded and (c) after Cu(II) loaded.

3.8. SEM and EDAX analysis

EDAX is one of the useful tools to evaluate the chemical and elemental characteristics of the adsorbent. In this study, SEM micrographs and EDAX spectra of *Bacillus* sp. (ATS-1) were taken before and after Pb(II) and Cu(II) sorption and presented in Figs. 11 and 12, respectively. SEM micrographs indicated that *Bacillus* sp. (ATS-1) surface was covered by a layer of Pb(II) looking like a sponge and Cu(II) ions looking like shiny bulky particles. This observation was confirmed by EDAX analysis which revealed Pb(II) and Cu(II) signals together with the presence of gold peaks in all spectra. The peaks at about 1.3 keV and between 3 and 4 keV corresponding to Mg^{2+} and K^+, respectively, disappeared after Cu(II) sorption. The disappearance of K^+ peaks between 3 and 4 keV and significant intensity decrease of Mg^{2+} peak at 1 keV were observed after Pb(II) sorption. These findings indicated that biosorption process also included ion-exchange mechanism for the both metal ions by this strain.

4. Conclusion

The results demonstrate that bacterial isolate *Bacillus* sp. (ATS-1) may be used as inexpensive, effective and easily cultivable biosorbent for the removal of Pb(II) and Cu(II) ions from aqueous solutions. The biosorption process of these metal ions on *Bacillus* sp. (ATS-1) was found to be dependent on experimental conditions such as the initial pH, initial metal ion concentration and contact time. The adsorption equilibrium data fitted well to Langmuir model for both metal ions in the studied concentration range. In competitive biosorption experiments the biosorption capacities of the biomass for metal ions decreased for one kind of ions with increasing the other metal ion concentrations. The interactions between metal ions and functional groups on the cell wall surface of the biomass were confirmed by FT-IR, SEM and EDAX analysis.

References

[1] B. Volesky, in: B. Volesky (Ed.), Biosorption of Heavy Metals, CRC Press, Boca Raton, 1990, pp. 7–43.
[2] P.X. Sheng, Y.P. Ting, J.P. Chen, L. Hong, Sorption of lead, copper, cadmium, zinc and nickel by marine algal biomass: characterization of biosorptive capacity and investigation of mechanisms, J. Colloid Interface Sci. 275 (2004) 131–141.
[3] A. Blanco, B. Sanz, M.J. Lama, L.J. Serra, Biosorption of heavy metals to immobilized *Phormidium laminosum* biomass, J. Biotechnol. 69 (1999) 227–240.
[4] H. Hussein, S. Farag, K. Kandil, H. Moawa, Tolerance and uptake of heavy metals by *Pseudomonads*, Process Biochem. 40 (2005) 955–961.
[5] G.M. Gadd, Heavy metal accumulation by bacteria and other microorganisms, Experientia 46 (1990) 834–840.
[6] B. Volesky, Z.R. Holan, Biosorption of heavy metals, Rev. Biotechnol. Prog. 11 (1995) 235–250.
[7] A. Kapoor, T. Viraraghavan, Fungal biosorption—an alternative treatment option for heavy metal bearing waste waters: a review, Bioresour. Technol. 53 (1995) 195–206.

[8] S. Pradhan, L.C. Rai, Biotechnological potential of *Microcystis* sp. in Cu, Zn and Cd biosorption from single and multimetallic systems, Biometals 14 (2001) 67–74.
[9] H. Liu, Y. Chen, Y. Lan, Y. Cheng, Biosorption of Zn(II) and Cu(II) by the indigenous *Thiobacillus thiooxidans*, Chem. Eng. J. 97 (2004) 195–201.
[10] G. Dönmez, Z. Aksu, Removal of chromium(VI) from saline wastewaters by *Dunaliella* species, Process Biochem. 38 (2002) 751–762.
[11] B. Volesky, Biosorbents for metal recovery, Trends Biotechnol. 5 (1987) 96–101.
[12] A. Kapoor, T. Viraraghavan, Heavy metal biosorption sites in *Aspergillus niger*, Bioresour. Technol. 61 (1997) 221–227.
[13] M. Ledin, Accumulation of metals by microorganisms—processes and importance for soil systems, Earth Sci. Rev. 51 (2000) 1–31.
[14] A. Malik, Metal bioremediation through growing cells, Environ. Int. 30 (2004) 261–278.
[15] O. Kandler, N. Weiss, Regular, nonsporing gram positive rods, in: P.H.A Sneath, N.S. Mair, M.E. Sharpe, J.G. Holt (Eds.), Bergey's Manual of Systematic Bacteriology, Williams & Wilkins, Baltimore, 1986, pp. 1208–1259.
[16] R.A. Slepecky, H.E. Hemphill, The genus *Bacillus* nonmedical, in: A. Balows, H.G. Trüper, M. Dworkin, W. Harder, K. Schleifer (Eds.), The Prokaryotes, 2nd ed., Springer-Verlag, Berlin, 1992, pp. 1663–1689.
[17] G.M. Gadd, Special microbial processes, in: H.J. Rehm (Ed.), Biotechnology, VCH, Verlagsgesselschaft, 1988, pp. 401–433.
[18] N. Kuyucak, B. Volesky, Biosorbents for recovery of metals from industrial solutions, Biotechnol. Lett. 10 (1988) 137–142.
[19] E. Fourest, C. Canal, J.C. Roux, Improvement of heavy metal biosorption by mycelial dead biomasses (*Rhizopus arrhizus*, *Mucor miehei*, and *Penicillium chrysogenum*): pH control and cationic activation, FEMS Microbiol. Rev. 14 (1994) 325–332.
[20] S. Tunali, I. Kiran, T. Akar, Chromium(VI) biosorption characteristics of *Neurospora crassa* fungal biomass, Miner. Eng. 18 (2005) 681–689.
[21] J.S. Chang, R. Law, C.C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, Water Res. 31 (1997) 1651–1658.
[22] A. Kapoor, T. Viraraghavan, D.R. Cullimore, Removal of heavy metals using the fungus *Aspergillus niger*, Bioresour. Technol. 70 (1999) 95–104.
[23] Z. Aksu, Equilibrium and kinetic modelling of cadmium(II) biosorption by *C. vulgaris* in a batch system: effect of temperature, Sep. Purif. Technol. 21 (2001) 285–294.
[24] M. Iqbal, R.G.J. Edyvean, Biosorption of lead, copper and zinc ions on loofa sponge immobilized biomass of *Phanerochaete chrysosporium*, Miner. Eng. 17 (2004) 217–223.
[25] H.M.F. Freundlich, Über die adsorption in lösungen, Zeitschrift für physikalische Chemie. 57 (1906) 385–470.
[26] I. Langmuir, The adsorption of gases on plane surfaces of glass, mica and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1403.
[27] Y. Sağ, A. Kaya, T. Kutsal, The simultaneous biosorption of Cu(II) and Zn(II) on *Rhizopus arrhizus:* application of the adsorption models, Hydrometallurgy 50 (1998) 297–314.
[28] Q. Li, S. Wu, G. Liu, X. Liao, X. Deng, D. Sun, Y. Hu, Y. Huang, Simultaneous biosorption of cadmium(II) and lead(II) ions by pretreated biomass of *Phanerochaete chrysosporium*, Sep. Purif. Technol. 34 (2004) 135–142.
[29] N. Friis, P. Myers-Keith, Biosorption of uranium and lead by *Streptomyces longwoodensis*, Biotechnol. Bioeng. 28 (1986) 21–28.
[30] C.P. Huang, C.P. Huang, A.L. Morehart, The removal of Cu(II) from dilute aqueous solution by *Saccharomyces cerevisiae*, Water Res. 24 (1990) 433–439.
[31] E. Fourest, J.C. Roux, Heavy metal biosorption by fungal mycelial byproducts: mechanism and influence of pH, Appl. Microbiol. Biotechnol. 37 (1992) 399–403.
[32] B. Mattuschka, G. Straube, Biosorption of metals by a waste biomass, J. Chem. Technol. Biotechnol. 58 (1993) 57–63.
[33] G. Yan, T. Viraraghavan, Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*, Water SA 26 (2000) 119–123.
[34] H. Ucun, Y.K. Bayhan, Y. Kaya, A. Cakici, O.F. Algur, Biosorption of lead(II) from aqueous solution by cone biomass of *Pinus sylvestris*, Desalination 154 (2003) 233–238.
[35] A. Selatnia, A. Boukazoula, N. Kechid, M.Z. Bakhti, A. Chergui, Y. Kerchich, Biosorption of lead(II) from aqueous solution by a bacterial dead *Streptomyces rimosus* biomass, Biochem. Eng. J. 19 (2004) 127–135.
[36] G.M. Gadd, L. De Rome, Biosorption of copper by fungal melanine, Appl. Microbiol. Biotechnol. 29 (1988) 610–617.
[37] F.Z. Kovačević, L. Sipos, F. Briški, Biosorption of chromium, copper, nickel and zinc ions onto fungal pellets of *Aspergillus niger* 405 from aqueous solutions, Food Technol. Biotechnol. 38 (2000) 211–216.
[38] A.Y. Dursun, G. Uslu, O. Tepe, Y. Cuci, H.I. Ekiz, A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrhizus* and *Aspergillus niger*, Biochem. Eng. J. 15 (2003) 87–92.